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DIFFERENTIAL NEUROSENSORY RESPONSES OF ADULT COLORADO POTATO BEETLE, *Leptinotarsa decemlineata*, TO GLYCOALKALOIDS

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Abstract—Neurons from chemosensory hairs on the galeae of adult Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), were investigated for responses to glycoalkaloids of the family Solanaceae. While solanine and tomatine elicited irregular firing by multiple neurons and bursting activity at 1 mM concentration in most sensory hairs, stimulation with leptine I resulted in consistently high-frequency, slowly adapting responses with a dose-dependent effect between 0.03 and 0.3 mM concentrations. Responses to a mixture of solanine and leptine I suggested possible modification of the leptine I response by other glycoalkaloids, resulting in reduced neural activity relative to leptine I alone. These results establish a method for specifically evaluating leptine I and other glycoalkaloids for effects on feeding behavior of CPB and provide a sensory component for incorporating deterrent chemistry into biorational control methods for the CPB.

Key Words—Gustation, taste, feeding deterrent, leaf beetle, potato, receptor neuron, glycoalkaloids, Colorado potato beetle, Chrysomelidae.

INTRODUCTION

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), is a major pest of potato, *Solanum tuberosum* (L.), throughout the world. Adaptability of the CPB and heavy use of synthetic pesticides for control has led to development of insecticide resistance in the insect and a shifting focus towards alternative,

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multifaceted control options. For example, information on feeding deterrents (Murray et al., 1996) and attractants (Dickens, 1999, 2000) could be incorporated into attracticide bait formulations and "push-pull" strategies of control, where insects are repelled by resistant or deterrent plants in combination with attracticide baits. Glycoalkaloids in potato have long been implicated as resistance factors against the CPB, specifically the leptines associated with *Solanum chacoense* Bitt. (Stürckow and Löw, 1961; Tingey, 1984), and breeding efforts have attempted to incorporate this natural resistance mechanism into control programs for CPB (Lawson et al., 1993; Tingey and Yencho, 1994).

Evidence continues to accumulate associating resistance with leptines. Significant differences between high- and low-leptine *Solanum chacoense* Bitt. clones for five resistance parameters (adult counts, adult feeding damage in the field, adult feeding on leaf disks, larval development, and larval survival) were directly related to concentration of foliar leptines in a study by Sinden et al. (1986). Sikinyi et al. (1997) showed reduced leaf area consumption and increased larval mortality on *S. chacoense* vs. *S. tuberosum*. Larval growth and development were reduced in a dose-dependent manner on artificial diets containing leptine I (Kowalski et al., 1999), although overall purity of the compound was only 60%.

Although there is evidence for a relationship between foliar leptine levels and resistance to CPB for *S. chacoense*, the mechanism or mode of action through which resistance is imparted has remained an open question. Stürckow and Löw (1961) first observed reduced feeding on potato foliage infused with leptine I. Tingey and Yencho (1994) labeled the leptines as potent antifeedants based on reduced feeding rates of adults on potato leaf disks from susceptible (*S. tuberosum* var. Kennebec) and resistant (*S. chacoense*) clones (from Sinden et al., 1986, 1988). Behavioral effects for adults and larvae in the field have been related to leptine levels alone among a number of glycoalkaloids (solanine, chaconine, leptinine I, and leptinine II) analyzed, indicating a feeding deterrent effect (Yencho et al., 2000). The relationship between the observed feeding effects and sensory detection of glycoalkaloids, however, has remained unclear. Leptines have been implicated as feeding deterrents and resistance has been attributed to the presence of these compounds, but these conclusions are based upon partial analysis of whole leaf chemistry. Since leptines of high purity have not been individually tested, the observed effects could be due to synergism with unknown components or other unidentified compounds in the foliage.

Harrison and Mitchell (1988) performed detailed behavioral observations on sampling and first meal feeding behavior of adult CPB on various host plants differing in glycoalkaloid content. Although they did not investigate *S. chacoense*, they questioned the assumption that glycoalkaloids act directly on the sensory system to inhibit feeding, raising the point that such conclusions have been based upon long-term feeding experiments. The possibility of postingestive effects on feeding behavior was discussed, as they found no inhibition of first meal feeding or

altered sampling behavior associated with the glycoalkaloid tomatine, concluding there was no evidence to indicate tomatine acted on the sensory system. The only studies investigating the effect of glycoalkaloids on CPB chemosensory neurons illustrated irregular firing by several neurons housed within sensilla on the tibia and tarsus (Stürckow, 1959) or the galea (Mitchell and Harrison, 1985; Mitchell, 1987). The firing pattern was often burstlike for glycoalkaloids, with the first burst of activity delayed by several seconds after stimulation. They concluded that there was no specialized chemoreceptor in CPB for the glycoalkaloids tested: solanine, chaconine, and tomatine. A survey of responses to seven other alkaloids (strychnine, caffeine, quinine, papaverine, sparteine, atropine, and arecoline) in adult CPB galeal chemosensilla also revealed no specific deterrent receptor for this class of compounds (Mitchell, 1987). There were no cases of phasic–tonic responses for these alkaloids, and bursting activity was observed with strychnine and quinine at concentrations of 5 and 10 mM. The specialized case of the leptines was discussed by Mitchell (1994), who indicated that despite the fact that no differential sensory effect among glycoalkaloids has been measured to date, the leptines deserved further study, as they had yet to be tested in sensory physiological assays.

Our objective here was to determine the nature of chemosensory responses to purified (100%) leptine I. We demonstrate for the first time specific chemosensory neural responses to a glycoalkaloid, leptine I, in adult CPB galeal taste hairs.

METHODS AND MATERIALS

Insects. Adult Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), were obtained from a colony with all life stages reared on *Solanum tuberosum* var. Kennebeck. Emerging adults were collected daily, sexed, and isolated in Petri dishes with moistened filter paper and fresh potato foliage that was replenished daily. Insects were kept in an environmental chamber (16L: 8D) at 25°C until use. Six-day-old insects were starved at least 4 hr prior to preparation for electrophysiological recordings.

Chemicals. Glycoalkaloids used in this study are illustrated in Figure 1. Tomatine (> 98% purity) and solanine (ca. 95%) were obtained from Sigma Chemical Co., St. Louis, Missouri. Leptine I was purified by using methodology modified from Kowalski et al. (2000).

Partially purified leptine I was obtained by normal-phase and reverse-phase chromatography from crude *Solanum chacoense* leaf extract. The sample was dissolved in methanol, and dissolution of the leptine I powder was aided by alternately heating a capped tube in a water bath, sonicating, and vortexing for a few seconds. A 1-ml aliquot of the sample was taken and transferred to a clean hydrolysis tube. The original sample tube was dried and stored in the cold for future use. Further

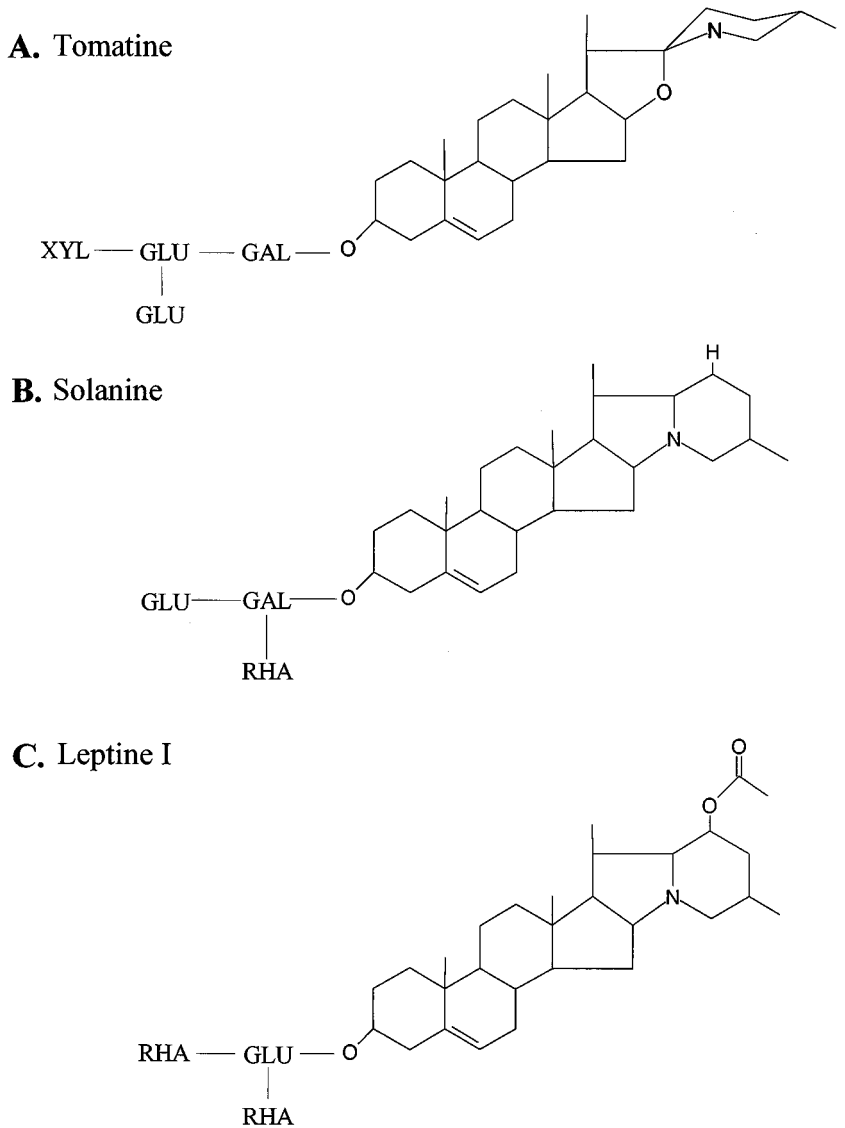

FIG. 1. Molecular structures of the glycoalkaloids used in this study: (A) tomatine, (B) solanine, and (C) leptine I. Abbreviations for sugar groups: GLU = glucose, GAL = galactose, XYL = xylose, RHA = rhamnose.

purification involved high-pressure liquid chromatography (HPLC) with a Waters 510 pump, Lambda-Max 481 wavelength detector, Waters automated gradient controller, Waters 745 data module, and a Waters 717 plus autosampler. A 20-μl volume of the leptine I aliquot was injected into a 3.9 $\times$ 300 mm Steel Resolve 5-μm spherical reverse-phase C_{18} column fitted with a Guard-pak C_{18} column insert to determine leptine I elution time and conditions for the fraction collector. HPLC condition was set at isocratic, with a mobile phase consisting of 1:1 acetonitrile (HPLC grade) –water supplemented with 5 ml 0.1 N phosphoric acid and 0.94 g 1-heptane sulfonic acid per liter. Flow rate was set at 0.7 ml/min and peak area detection was at 205 nm. Based on this initial injection, leptine I elution occurred about 8–9 min after sample injection. Thus, a Gilson FC 205 fraction collector in line with the HPLC was programmed to collect leptine I fractions from 8 and 12 min after sample injection. Fractions were eluted at a rate of 0.28 min per collection tube. Sample injections were done in 20-μl aliquots. Aliquots (5–10 μl) from these fractions were spotted on a Whatman flexible-backed polyester silica plate. Leptine I was visualized by spraying the plate with 0.11 M potassium iodide and 0.6 mM bismuth subnitrate in 3.5 M acetic acid (Dragendorff's spray reagent). Fractions containing leptine I were individually transferred to a new and clean test tube. The total volume in each tube was marked with a pen, and each tube was dried down to half the volume by blowing off acetonitrile. Acetonitrile was evaporated in the fume hood by gently heating the tubes in a beaker containing water set on a hot plate and by applying air into each tube while heating. The remaining solution from each tube was subjected to a final solid-phase extraction by using a vacuum manifold and classic C_{18} Sep-pak cartridges. This process involved conditioning the cartridges with 3 ml methanol followed by 5 ml of the aqueous heptane sulfonic acid extraction reagent. The fractions were loaded on to each conditioned Sep-pak cartridge, followed by a 5-ml acetonitrile: water (20:80) rinse. Leptine I was eluted with 2 ml methanol. Quantification and purity of leptine I was determined by analytical HPLC. α-Solanine (0.1 mg/ml methanol) served as a standard.

Solutions of glycoalkaloids were prepared for electrophysiology with 0.01 M NaCl as reference. Glycoalkaloids were solubilized in 0.01 M NaCl with lowered pH ($\sim$2–3) with HCl, then brought to pH 5.2–6.3 with NaOH. Reference electrolyte (0.01 M NaCl) was treated similarly.

Electrophysiology. Responses of galeal chemosensilla were obtained by using a standard tip-recording technique (Hodgson et al., 1955). A whole body preparation was used with adult CPB affixed inverted on cork with tape, and the head was immobilized with a tungsten collar. The head was pulled to a prognathous position with a tungsten post pressed into the cork that hooked and immobilized the mandibles. A galea was then pulled laterally with a fine nylon thread roped around the maxillary palp and immobilized by pinning between two fine

tungsten needles inserted into the cork. Use of a whole-body preparation and restriction of insect movement allowed us to identify individual sensilla and record from most, if not all ~16 chemosensory sensilla present on individual galea. A sharpened tungsten electrode inserted into the abdomen of the insect served as a reference electrode. Silver wire inserted into a glass capillary, pulled and sized to fit over the tip of one galeal chemosensory hair and filled with treatment chemicals in 0.01M NaCl, served as a stimulating/recording electrode. Both electrodes were connected to a Grass P15D AC amplifier. Chemosensory responses were viewed on an oscilloscope, monitored with a loudspeaker, and digitized for storage and analysis on a computer with Sapid (Smith et al., 1990) and AutoSpike (Syntech, Hilversum, The Netherlands) software. Numbers of nerve impulses within the first 500msec following the onset of stimulation were counted for analyses.

Experimental Protocol. In the first experiment, individual glycoalkaloids and 0.01 M NaCl reference stimulus treatments were tested successively on 5–16 hairs per galea. At least 3 min were allowed between stimulation of individual hairs. Concentrations of glycoalkaloids tested were 0.01, 0.1, and 1 mM for both male and female CPB. Leptine I concentrations tested also included 0.03 and 0.3 mM for construction of an expanded dose–response curve for female CPB. All leptine I concentrations were tested on at least 18 hairs from two to four female CPB galea, with the exception of 0.03 mM (10 hairs from one female CPB). For 1 mM solanine, 70 hairs from five females and three males were recorded; for 1 mM tomatine, 60 hairs from four females and two males were sampled. Contact with sensory hairs was maintained for at least 30 seconds to detect any glycoalkaloid-induced bursting of sensory neurons. Time to first burst was noted, and stimulation ended after a 60-sec period.

The second experiment investigated the effect of solanine on responses to leptine I. Treatments for this experiment were tested in the following order: 0.01 M NaCl, 0.3 mM leptine I (peak of dose–response curve), 1 mM solanine, 0.3 mM leptine I + 1 mM solanine, 0.3 mM leptine I. Records were obtained from nine hairs for one female CPB.

One-way analysis of variance was performed on log $(x + 1)$ transformed data to test for treatment effects on nerve impulse frequency. Duncan's multiple range test at 5% confidence level was used to test for differences between treatment means (Snedecor and Cochran, 1967).

RESULTS

Of the three solanaceous glycoalkaloids tested, only leptine I elicited a normal chemoreceptor response from a single neuron (Figure 2B). Responses increased

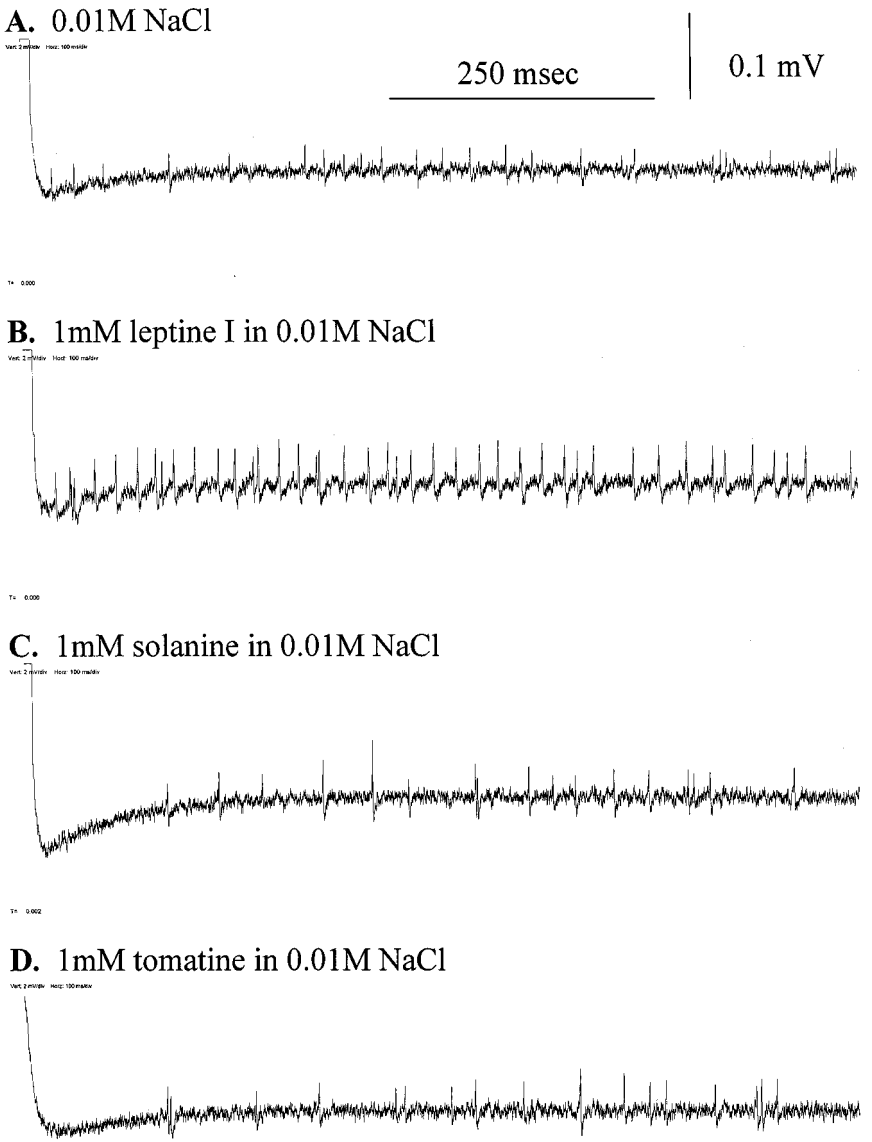

FIG. 2. Neural responses from chemosensory hairs on adult CPB maxillary galea to glycoalkaloids. Traces represent 1 sec after stimulation of sensory hair with: (A) 0.01 M NaCl reference solution, and (B) 1 mM leptine I, (C) 1 mM solanine, and (D) 1 mM tomatine, all in 0.01 M NaCl.

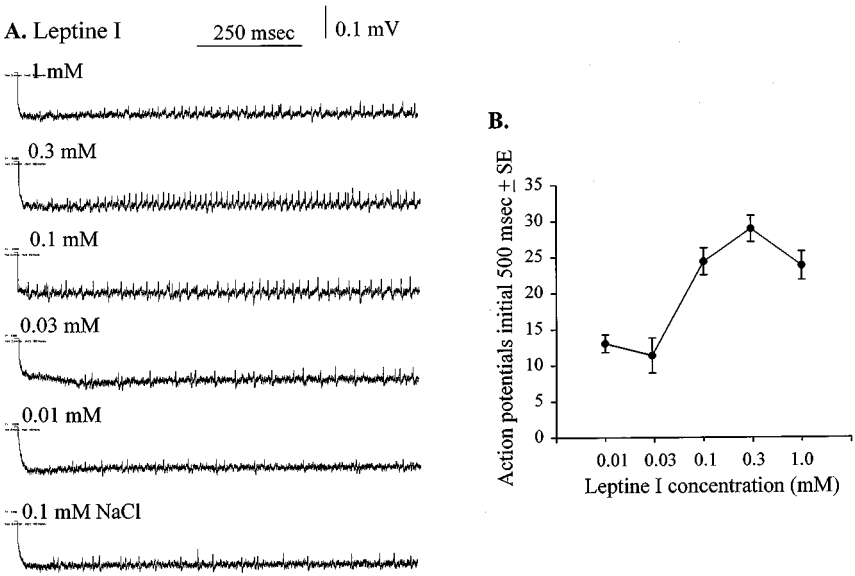

FIG. 3. (A) Representative neural responses to increasing concentration of leptine I. Traces represent 1 sec after stimulation of sensory hair with leptine I. (B) Dose-response curve for adult female CPB galeal chemosensory neurons in response to increasing concentrations of leptine I in 0.01 M NaCl. Action potentials were counted for the initial 500 msec following stimulation. $N = 10$–44 replicates/dose.

in a dose-dependent manner (Figure 3A and B), with a threshold at or above 0.03 mM and maximal response at 0.3 mM concentration. Leptine I did not elicit bursting activity below 1 mM concentration; at 1 mM weak bursting activity was observed in 9% (3/35) of hairs recorded. Percent of hairs stimulated responding to the leptine I concentrations were 12% (3/25) at 0.01 mM, 50% (5/10) at 0.03 mM, 88% (22/25) at 0.1 mM, 100% (18/18) and 83% (29/35) for 0.3 mM and 1 mM leptine I, respectively.

Two glycoalkaloids closely related to leptine I, solanine and tomatine, did not elicit responses greater than the control (Figure 2C and D), with bursting of multiple neurons (spikes of two or three amplitudes) observed only with the 1 mM treatments (Figure 4A and B). Bursting did occur within 60 sec in 53% (42/79) of hairs tested with 1 mM solanine, with an average delay of 13 sec. For 1 mM tomatine, bursting occurred in 32% (19/60) of hairs, with an average delay of 29 sec.

Although solanine has no stimulatory effect on this chemoreceptor neuron, it does have a depressive effect on this neuron's response to leptine I that lasts for at least 3 min (Figure 5).

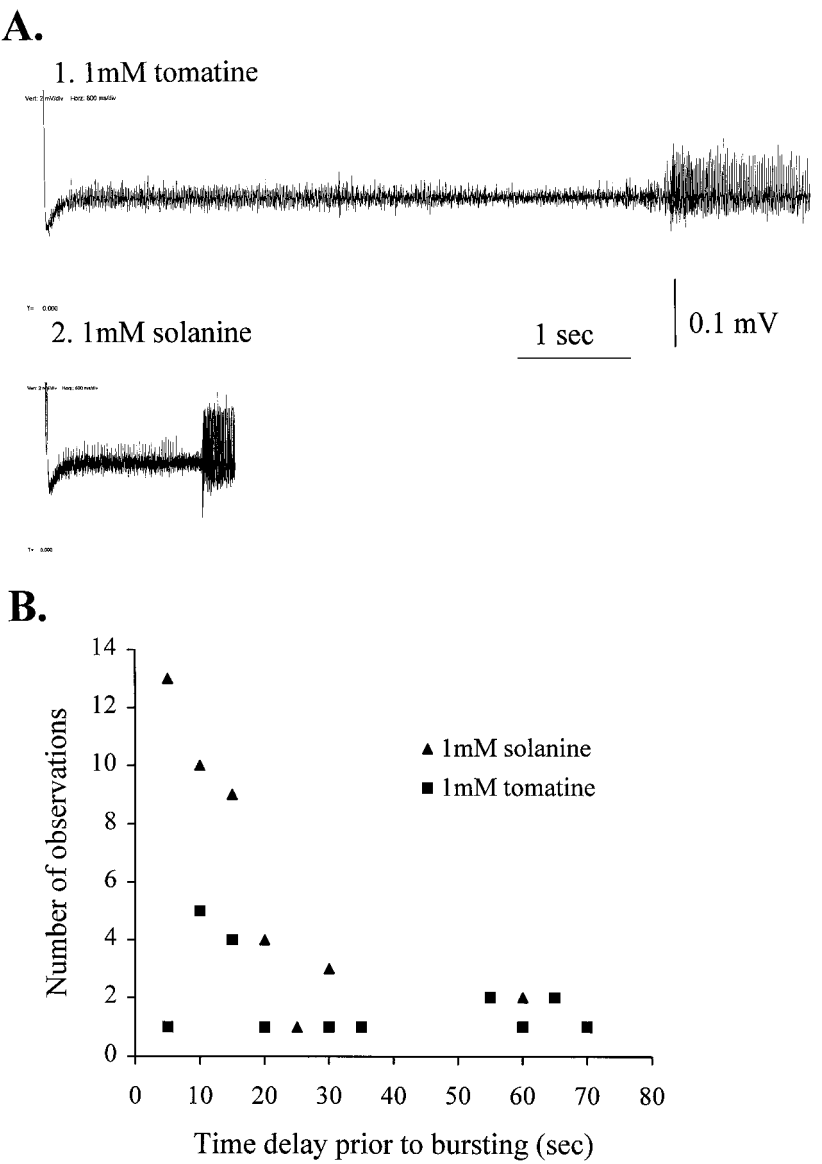

FIG. 4. Bursting response of neurons within adult CPB galeal chemosensory hairs. (A) Neural responses to 1 mM tomatine in 0.01 M NaCl and 1 mM solanine in 0.01 M NaCl illustrating bursts of chemosensory neurons. (B) Plot showing delay times before bursting activity for solanine and tomatine stimuli.

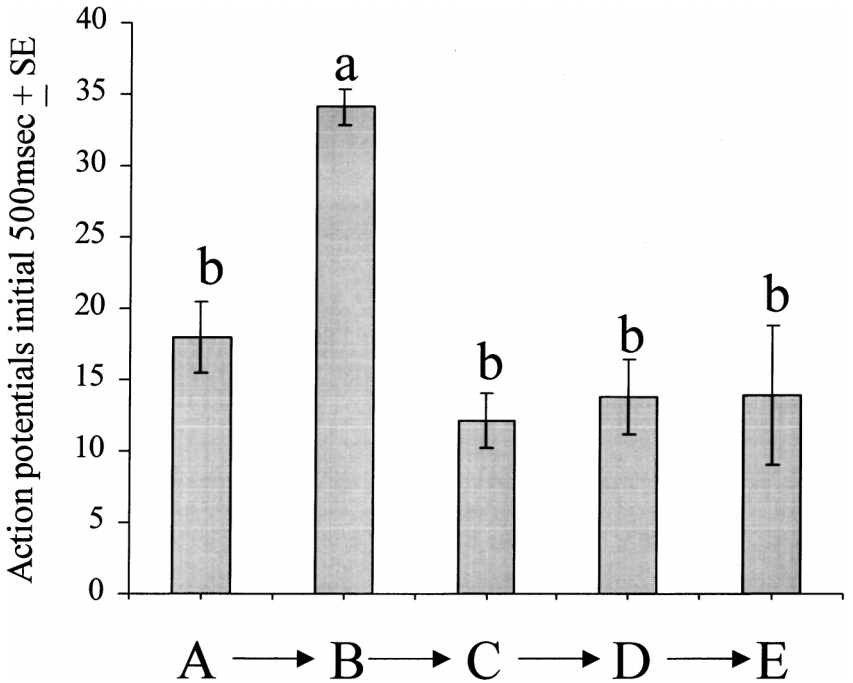

FIG. 5. Mean neural responses from adult female CPB galeal chemosensory hairs to sequential stimuli. Treatments were applied to individual hairs in the following order with three minutes between treatments: (A) 0.01 M NaCl, (B) 0.3 mM leptine I, (C) 1 mM solanine, (D) 0.3 mM leptine I + 1 mM solanine, and (E) 0.3 mM leptine I. $N = 9$ hairs/treatment. Different letters above the bars indicate significant differences between treatment means at the $\alpha = 0.05$ level.

DISCUSSION

We report here the first recordings of a specific chemosensory neural response to a glycoalkaloid in CPB. This slowly adapting response to leptine I is largely of a single neuron and contrasts with the lack of response to two other host plant glycoalkaloids, solanine and tomatine, on adult CPB chemosensory hairs.

The main differences in the three molecules tested here lie in the acetyl group at C-23 of the glycoalkaloid carbon skeleton in leptine I, as well as in the sugar group (Figure 1). Perhaps the differential effect observed is a function of different solubilities of individual glycoalkaloid molecules, with leptine I more readily solubilized and available at the receptor site. The more amphipathic tomatine and solanine may dissolve into and disrupt the dendritic membrane and thereby

elicit sporadic firing of several neurons and bursts of nerve impulse activity in adult CPB galeal taste hairs.

Specialist deterrent chemoreceptors identified for several species of larval Lepidoptera (Schoonhoven, 1982) respond to the alkaloids quinine and strychnine, as well as azadirachtin. In *Pieris* spp., receptors sensitive to cardenolides (threshold of 0.1–0.3 μM) occur in both adults (Stadler et al., 1995) and larvae (van Loon and Schoonhoven, 1999). These compounds are present in cruciferous plants and deter both feeding and oviposition. Such deterrent receptor neurons have only recently been reported for beetles in the family Chrysomelidae. Chyb et al. (1995) showed chemosensory responses in adult western corn rootworm (*Diabrotica virgifera* LeConte) to the feeding deterrents β-hydrastine and strychnine. They also found chemosensory responses to cucurbitacin B isolated from host plants in the family Cucurbitaceae. Cucurbitacins, strong feeding deterrents for most insects, act as potent arrestants and feeding stimulants for western corn rootworm and a small group of closely related species. Messchendorp et al. (1998) reported a deterrent receptor in CPB larvae in epipharyngeal sensilla that respond to the glucosinolate, sinigrin, and a synthetic sesquiterpene analogue, drimane, with threshold concentrations of 0.01 and 0.1 mM, respectively. Maximal numbers of action potentials to drimane were in response to 1 mM. Our observations of the first deterrent receptor neuron in adult CPB reveal a similar response range between 0.03 and 0.3 mM for leptine I. Both sinigrin and the drimane analog are feeding deterrents, but are isolated from non host plant species from the families Cruciferae and Polygonaceae, respectively. Adult CPB response to leptine I is similar to the *Pieris* spp. response to cardenolide in one respect, namely, both are deterrent receptor neuron responses to particular compounds from unacceptable plants in their own host-plant families.

Glycoalkaloids and other deterrents reduce neuronal responses to chemicals that stimulate feeding in CPB. Response to 10 mM γ-aminobutyric acid (GABA) was completely abolished following stimulation of the same hair with 0.5 mM solanine for 20 sec (Mitchell and Harrison, 1984); 1 mM tomatine, papaverine, and sparteine also significantly reduced this response (Mitchell, 1987). Response to 10 mM sucrose was similarly inhibited by 1 mM quinine and papaverine (Mitchell, 1987). Solanine and tomatine suppress neuronal response to (*E*)-2-hexenol, which Mitchell and McCashin (1994) suggest stimulates the same cell as sucrose, alanine, and GABA in some adult CPB galeal chemosensilla. Messchendorp et al. (1998) conclude that drimane inhibits the response of a sucrose cell in larval CPB epipharyngeal sensilla. Schoonhoven (1982) discussed the perception of antifeedants at the sensory level and identifies at least two possible mechanisms: (1) stimulation of specialized receptors by feeding deterrents, or (2) modification of the activity of receptors responding to feeding stimulants. Previous reports provide evidence for the latter mechanism of feeding deterrence. Our observation of sensory neurons

responsive to leptine I provides evidence for both mechanisms. Interestingly, here we also show suppression of a deterrent sensory neuron responsive to submillimolar levels of leptine I by millimolar levels of another glycoalkaloid, solanine, for which no specific taste receptor was identified. Thus, glycoalkaloid effects may be modulated by related compounds, and such interaction effects among the complex mixture of chemicals in host plant foliage must be considered when drawing conclusions about behavioral effects and mechanisms of host-plant selection.

Responses to solanine and tomatine were consistent with those described by Mitchell and Harrison (1985) and Mitchell (1987) for chemosensory neurons within galeal sensilla and by Stürckow (1959) for neurons within sensilla on the palps and tarsi. In both cases, the responses consisted of irregular firing of several neurons and bursting activity after a delay of several seconds. Discovery by us of an identifiable sensory neuron for leptine I provides a mechanism by which glycoalkaloids of the Solanaceae are detected by CPB. Our results not only establish a neural pathway for detection of leptine I but also provide a mechanism of feeding deterrence and resistance to CPB feeding attributed to this glycoalkaloid (Stürckow and Lüw, 1961; Tingey, 1984; Sinden et al., 1986). Previous experiments to determine the effects of leptines on feeding by CPB have either involved correlation of reduced consumption of foliage with levels of leptine, or feeding tests with leptine of partial (60%) purity. Feeding bioassays with purified leptine I coupled with electrophysiological observations will establish input–output relationships to more conclusively relate behavioral effects of leptine I to neural responses of identified chemoreceptor neurons.

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